Journal of
TRAVEL MEDICINE

BRIEF COMMUNICATION

Underdiagnosis of Chikungunya Virus Infections in Symptomatic Dutch Travelers Returning From the Indian Ocean Area

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DOI: 10.1111/j.1708-8305.2012.00670.x

A putative underdiagnosis of clinical chikungunya virus infection in Dutch travelers to the Indian Ocean area was addressed by retrospective screening of all sera for which requested dengue virus serology was negative in the period 2007 to 2010. Evidence for a recent infection was observed in 6.5% of 107 patients, indicating a substantial underdiagnosis and the need for increased awareness among physicians.

Dengue virus (DENV) is a major cause of fever in travelers returning from Southeast and Central Asia. Since 2004, chikungunya virus (CHIKV) has emerged as an important cause of fever in travelers to the Indian Ocean islands and India as well, and this virus has spread to Southeast Asia.[1] Both DENV (genus *Flavivirus*, family Flaviviridae) and CHIKV (genus *Alphavirus*, family Togaviridae) are transmitted to humans by mosquitoes. The principal vector for both DENV and CHIKV transmission is *Aedes aegyptii*, which is omnipresent in tropical and subtropical regions of the earth. Another important common vector is *Aedes albopictus*, which has expanded its geographic distribution from Asia to Southern Europe, the Americas, and parts of Africa and Australia through international trade in used tires. It has been the primary vector in many of the recent CHIKV outbreaks.[1,2] The establishment of *A albopictus* in Southern Europe in the last decade has enabled a substantial outbreak of autochthonous CHIKV transmission in Italy in 2007 (>200 laboratory-confirmed cases), autochthonous DENV and CHIKV transmission in France in 2010, and autochthonous DENV transmission in Croatia in 2010. These viruses were introduced in Europe through viremic travelers returning from endemic countries.[1,2]

Given the overlapping geographic distribution of DENV and CHIKV, the possibility of a CHIKV infection should be included in the differential diagnosis of febrile illness with rash within 2 weeks of return from endemic areas.[1,3,4] However, in practice, physicians requesting diagnostic work-ups may not be aware of this disease, especially in nonendemic countries like the Netherlands where physicians have little experience with CHIKV.

The aim of this study was to address this putative underdiagnosis of clinical CHIKV infection in Dutch travelers to the Indian Ocean region by retrospective screening of sera of patients for which requested DENV serology was negative in the period 2007 to 2010. In addition, the trends in diagnostic requests for CHIKV and DENV infections related to travel to the Indian Ocean area were analyzed.

Methods

The sera were tested in serial (two-step) dilutions using a commercial indirect immunofluorescence assay (IFA, Euroimmun, Lübeck, Germany) for IgG and IgM anti-CHIKV. This IFA was proven to be suitable in outbreaks of both A226 and A226V envelope protein E1 variants of East, Central, and South African genotype CHIKV, both known to have replaced the

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Asian CHIKV genotype in India and Southeast Asia and responsible for the Indian Ocean area outbreaks.[5,6] Because background reactivity was observed in a high proportion of serum samples of non-travelers in dilutions up to 1 : 40 to 1 : 80 (data not shown), serum samples with a positive signal at dilution 1 : 128 or higher were considered to be positive. For serum samples that were reactive at dilution 1 : 64, repeat sampling was requested. Given the rather high proportion of samples with some nonspecific background reactivity, sera testing positive were analyzed for independent confirmation of the results using a virus neutralization test with vital staining (NT)[7] using CV-1 cells and the prototype East, Central, and South African lineage CHIKV strain S27-African. The different CHIKV lineages and strains are known to cross-neutralize.[6] In addition, sera were analyzed for the presence of CHIKV RNA using a Taq-Man reverse-transcription polymerase chain reaction (PCR; Roche Diagnostics, Almere, The Netherlands) as described in Ref. (8). PCR and NT were only performed when volume of remaining serum was sufficient.

We selected serum samples from unique patients that had been submitted for diagnosis of DENV infection in 2007 to 2010 ($n = 948$) and for whom minimal background data had been provided including travel destination ($n = 348$, 36.7%). From this group, we selected the patients who had traveled to the Indian Ocean region ($n = 158$). To this goal, all countries with a coastline on the Indian Ocean were included. Subsequently, patients with positive DENV serology (IgM and/or IgG; $n = 41$, 25.9%) or inconclusive DENV serology (a-specific reaction, $n = 5$) were excluded. Of the remaining 112 patients, sera of 107 patients were available for CHIKV serology.

Results

A total of 107 sera from travelers returning from destinations in the Indian Ocean area, showing clinical symptoms corresponding to a DENV infection but with negative DENV serology, were analyzed for the presence of CHIKV IgM and IgG. The patient cohort consisted of 63 males and 44 females, ranging in age from 1 to 78 years with a median age of 36, and traveling to Indonesia (36.5%), Thailand (35.5%), India (10.3%), Malaysia (3.7%), Tanzania (3.7%), South Africa (3.7%), Sri Lanka (2.8%), Mozambique (1.9%), Australia (1.9%), and Malawi, Dubai, Mauritius, Kenya, Singapore, Oman, Bahrain, Iran, and United Arab Emirates (all 0.9%). Twenty-two patients had traveled in 2007, 39 in 2008, 23 in 2009, and 23 patients in 2010.

Antibodies to CHIKV were detected in sera of eight travelers (7.5%; Table 1). Seven patients had clear evidence of recent infection (6.5%), based on both IgM- and IgG-positive serology ($n = 5$), or IgM serology confirmed by PCR and/or NT ($n = 2$). A second serum sample of one of the two IgM-positive patients showed seroconversion for IgG. One traveler was only IgG positive at a single time point 25 days upon return from the Indian Ocean area. As CHIKV IgM are typically lasting up to 3 months postinfection, this patient probably had prior exposure to CHIKV unrelated to the current complaints for which DENV diagnostics were requested. Of the seven travelers with chikungunya, three had visited Thailand, one had a history of travel to both Thailand and Malaysia, two had traveled to Indonesia, and one to India (Table 1). In total, 6.3% of the male patients and 9.1% of the female patients of the cohort showed evidence of a CHIKV infection. The neutralization assay confirmed the presence of CHIKV-specific antibodies in sera of four out of four patients with acute symptoms. For five seropositive travelers, the remaining amount of serum was insufficient to perform a NT (data not shown).

Discussion

This study demonstrates that in the Netherlands CHIKV infections were substantially underdiagnosed in travelers suspected of dengue and returning from the Indian Ocean area in the period 2007 to 2010. In 6.5% of the travelers with negative DENV serology, CHIKV appeared to be the etiologic agent. For comparison, of the total of 158 travelers to this region for whom DENV diagnostics were requested, 25.9% showed a positive serology for DENV IgM and/or IgG. As coinfections of humans with DENV and CHIKV have been described, the results of this study potentially underestimate the number of CHIKV cases. Some of the DENV positives could have been coinfected with CHIKV.[2] An analysis of air passenger traffic from CHIKV hotspots to Europe resulted in an estimated annual number of 1,302 viremic travelers from India and Malaysia to the Netherlands,[9] supporting our observation that CHIKV infections are underdiagnosed in the Netherlands as only three CHIKV infections were diagnosed in our laboratory in the period 2009 to 2011 (data not shown). Recently, a similar observation of underdiagnosis was described for Germany.[10]

Although infections with DENV or CHIKV are both typified by fever, myalgia, and rash, and the reported incubation periods are similar (4–7 d for DENV, range 3–14 d; 3–7 d for CHIKV, range 1–12 d), other clinical features are clearly different. A CHIKV infection is typified by arthritis, tenosynovitis, and (prolonged) arthralgia. Dengue fever is typically associated with retro-orbital pain, minor bleeding, and hypotension.[1,3,4] The absence of an experienced "eye" for these differences besides the overlapping clinical manifestations in combination with an insufficient awareness for CHIKV as a possible cause for infection might explain the observed underdiagnosis of CHIKV. The Netherlands is a nonendemic country and the physicians (both general practitioners, who give the first line of care, and infectious disease specialists) are not confronted with CHIKV on a regular basis, thereby potentially overlooking CHIKV in their differential diagnosis of travel-related fever.

Table 1 CHIKV-positive travelers with negative DENV etiology returning from Indian Ocean area to the Netherlands in the period 2007 to 2010

Patient ID	Travel destination	Year of travel	Sex	Age	CHIKV IgG*	CHIKV IgM*	PCR	Conclusion
1	Indonesia	2007	M	61	1 : 4,096	1 : 256	nt	Pos.
2	Indonesia	2007	F	59	1 : 2,048	1 : 4,096	Neg.	Pos.
3†	Thailand	2009	F	28	Neg.	1 : 1,024	Neg.	Pos.
4	India	2009	M	50	1 : 4,096	1 : 2,048	Neg.	Pos.
5‡	Indonesia	2009	M	67	1 : 1,024	Neg.	Neg.	Past exposure
6§	Malaysia, Thailand	2009	M	58	Neg.	1 : 2,048	Pos.	Pos.
					1 : 2,048	1 : 1,024	Neg.	Pos.
7	Thailand	2009	F	29	1 : 4,096	1 : 512	nt	Pos.
8	Thailand	2010	F	26	1 : 2,048	1 : 256	nt	Pos.

CHIKV = chikungunya virus; DENV = dengue virus; PCR = polymerase chain reaction; nt = not tested; Pos. = positive result; Neg. = negative result.
*Highest dilution reactive. Scored negative (Neg.) when reactive dilution is ≤1 : 32. When reactive dilution is ≤1 : 64 a follow-up serum is requested in routine diagnostic setting.
†Serum taken within 1 week of the onset of symptoms.
‡Serum taken 25 days upon return from Indian Ocean area.
§Paired serum sample. Second serum taken 4½ weeks after first serum.

Patients with febrile illness returning from regions endemic for DENV and CHIKV should be evaluated by default for both pathogens. This situation could be addressed by offering only combined testing for CHIKV and DENV for travelers to regions where both viruses circulate (Africa and Indian Ocean area), whereas single DENV testing is offered for regions where CHIKV is not known to circulate (the Americas, Caribbean). However, one might argue for combined testing in geographic regions where CHIKV is not known to circulate but competent vectors are present (for instance, all DENV-endemic regions). The cases of autochthonous CHIKV transmission in Europe and its fast geographic expansion into Southeast Asia illustrate the dynamic nature of spread of arbovirus infections. CHIKV could be introduced into new regions including the Americas and the Caribbean. This study also illustrates the lack of information on travel destination in diagnostic requests. Only 36.7% and 41.9% of the respective DENV and CHIKV requests provided information on travel destinations. This lack of information and the higher costs for combined diagnostics might complicate the implementation of this diagnostic algorithm in diagnostic laboratories. Furthermore, the omission of travel destination information in the majority of diagnostic requests complicates the use of travelers as sentinels to identify unknown regions with virus circulation as was recently shown for Africa.[2]

In conclusion, an increased awareness among physicians in the Netherlands for CHIKV appears indicated and would also be a prerequisite for timely detection of potential autochthonous cases as the main vector species *A albopictus* and *A aegyptii* are repeatedly introduced into the Netherlands through the trade in used tires.

Acknowledgments

M. Kuijer and N. Cleton are acknowledged for technical assistance and critical reading of the manuscript.

Declaration of Interests

The authors state that they have no conflicts of interest.

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